Supercritical Carbon Dioxide Sterilization of Ultra-High Molecular Weight Polyethylene

HONORS THESIS

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Abstract

The aim of this research was to use a benign technique for the sterilization of ultra-high molecular weight polyethylene (UHMWPE), which is broadly used in artificial joints and other medical implants. Current sterilizations methods such as gamma irradiation, ethylene oxide gas, and gas plasma can damage the polymer, thus reducing its service life, as well as create harmful byproducts. Supercritical carbon dioxide (SC) CO_2 sterilization has the potential to greatly reduce both polymer degradation and negative environmental impacts. The former extends the life of the implant and the latter decreases waste disposal costs and minimizes risk to sterilization workers. The feasibility of using SC CO_2 modified with additives such as ethanol, water and hydrogen peroxide was assessed for the sterilization of UHMWPE. The operating conditions and the amount of modifiers were optimized to achieve a complete inactivation of bacteria (spores included) and fungi. When equal volumes of all modifiers were added to CO_2 at 37ºC and 170 bar so that their total mole fraction was $\geq$ 4.1E-03, complete inactivation of all microorganisms was achieved within 2 hours. The physio-chemical properties of the polymer were tested for untreated, as well as treated samples. An Instron measured the mechanical strength and elongation of the polymer, and Fourier Transform Infrared (FTIR) spectroscopy was used to measure the level of oxidation of the polymer to evaluate polymer chain damage. Both the physical and chemical properties of the polymer were unchanged after the SC CO_2 sterilization technique. This research has shown that it is possible to sterilize medical grade polymer, under relatively safe conditions, without affecting its physio-chemical properties.

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1. Introduction

1.1 Background

Throughout the Middle Ages and into the 1800's, diseases were thought to be caused by a miasma or "bad air". People felt the spread of diseases could be prevented by merely avoiding toxic smells or decaying material.[1] Surgeons would commonly go from performing autopsies to delivering a child and then back again without so much as rinsing their hands. Similarly unhealthy practices continued within the medical profession until the late 1800's when the germ theory of disease gained credibility by explaining the increased cases of illnesses within hospital settings.[2] Since then, hygienic practices and sterilization methods have been used to drastically decrease the spread of disease and infections.

One of the first of these sanitation practices was the use of carbolic spray for surgical sterilization in 1867.[3] Unfortunately, carbolic spray was caustic to skin and body tissues, which prevented the use of it in large quantities.[4] Throughout the 1880's and 1890's antiseptic surgical dressings and other forms of sterilization such as dry-heat and steam pressure were introduced to the medical field. The advent of suture sterilization as well as the aforementioned sterilization procedures quickly dropped the surgical death rate (due to infection and disease) from 40% to less than 3%.[5] The passage of time and the increased development in technology led to the first use of ethylene oxide gas as a hospital sterilant in 1940, radiation sterilization in 1956, gamma radiation sterilization in 1964, and gas plasma sterilization in 1993. The advancements in variety and efficacy of sterilization processes, along with the development of antibiotics and better surgical

procedures, have increased the life expectancy in well developed countries to ~78 years.[5] This extended longevity leads to an increased duration of wear and tear on the body that inevitably leads to partial or total breakdown of overused joints and articulating surfaces. As time passes and medical knowledge advances, the once main focus of medicine, "quantity of life" comes second to the ever expanding "quality of life" issue.

The advent of implantable prosthetics has come a long way in alleviating the quality of life issue by replacing worn out joints; but there is still vast room for improvement. While the materials used to make implants are becoming increasingly more suitable for *in vivo* use, there are still issues in regards to sterilization. Sterilization techniques were designed specifically for *ex vivo* materials and surgical instruments that were not intended to be left in the body. Sterilization techniques must be re-evaluated to insure they work effectively on implants and do not have any lasting harmful affect on the properties of the implant or the tissue that surrounds it. Current research has shown that the type of sterilization can have an effect on the durability and *in vivo* lifespan of the implant.[6] In order to accommodate the increasing longevity of humans, new sterilization techniques that do not have harmful effects on implantable devices must be discovered and evaluated.

1.2 Problem Statement

The purpose of this study was to assess the feasibility of using supercritical carbon dioxide and small amounts of chemical modifiers to reduce bacterial and fungal contaminations deposited onto ultra-high molecular weight polyethylene to acceptable FDA standard levels. A 6 log reduction of bacterial spores is required by the FDA in order to approve any sterilization technique.[7] The chemical modifiers used in conjunction with supercritical carbon dioxide (SC CO_2) in this study were ethanol, distilled water, and hydrogen peroxide. These modifiers are known to play an important role in the sterilization procedure and as such the volume of each modifier was varied in order to determine the their relative importance in the sterilization process as well as what conditions resulted in a 6 log reduction while minimizing the total mole fraction of modifiers.

It is also important to demonstrate that the SC CO_2 sterilization process had no significant impact on the mechanical strength or oxidation level of the polymer implants fabricated from UHMWPE. As any sterilization process may have a negative impact on the sterilization media, it is important to determine the degree of degradation so the sterilization process can be evaluated for implant applicability.

1.3 Statement of Objectives

The three main goals of this research were:

1) To determine optimal conditions that would ensure complete sterilization (6 log reduction) of all bacteria (including spores) and fungi while minimizing the amount of a required additives.

2) To determine the effect of the sterilization process on the mechanical strength of treated ultra-high molecular weight polyethylene.

3) To determine the effect of the sterilization process on the level of oxidation of treated ultra-high molecular weight polyethylene.

2. Literature Review

2.1 Ultra-High Molecular Weight Polyethylene

Approximately 570,000 total hip and knee replacement surgeries are performed every year that use ultra-high molecular weight polyethylene (UHMWPE) as the key articulating surface componenent.[8] This number is expected to grow to 750,000 total replacement surgeries by 2030.[9] Since 1995, the market for medical implants has grown from 1.8 billion dollars to 3.5 billion dollars in 2004 and has a current revenue growth rate of 12%.[10,11] The growing need for implants, and subsequently UHMWPE components, mirrors an increasing desire to better understand how various pre-implantation polymer treatments affect its desirable high tensile strength, high impact strength, and corrosion and abrasion resistant properties. The main problem with UHMWPE implants is the *in vivo* creation of small UHMWPE particulates due to normal wear of the implant. Unfortunately, these particles activate an immune response which leads to chronic inflammation and ultimate failure of the implant. Recent research has shown that the method of sterilization can have a large affect on the the *in vivo* longevity of the implant.

2.2 Commercial Sterilization Methods for UHMWPE

2.2.1 Gamma Irradiation

Gamma irradiation has been used to sterilize UHMWPE components since the 1960's and is still largely used in industry today. Typically, the polymer samples are treated with a radiation dosage of 25 to 40 kGy in order to fully sterilize them. The samples were then originally stored in an air-permeable package but this was found to be

detrimental to the implant. The gamma irradiation sterilization process creates large quantities of free radicals (from polymer chain scission) throughout the polymer, which become oxidized when exposed to air.[12] This oxidation process increases the crystallinity and density of polymer and leads to decreased mechanical strength properties. Most noticeably is the increased embrittlement of the region 1-2 mm below the articulating surface of the polymer. The irradiation can also cause crosslinking of the polymer chains which may improve the mechanical properties. Industry standards have since switched over to using non-permeable packing for storage of the polymer samples.

2.2.2 Ethylene Oxide

Ethylene oxide has been used to sterilize UHMWPE since the 1980's and while it works well against bacteria, spores, and viruses, it is highly toxic. Ethylene oxide is a good candidate for UHMWPE sterilization because the polymer does not chemically react with ethylene oxide. During sterilization, the temperature, duration, and humidity must be rigorously controlled but this process does effectively sterilize entire modular components without affecting the physical, chemical, or mechanical properties of UHMWPE. Besides the high levels of toxicity, the largest draw back with using ethylene oxide as a sterilization method is the cycle time. The process begins with an 18 hour period of preconditioning with 65% relative humidity and is then followed by 100% ethylene oxide gas exposure at 0.04MPa. The UHMWPE samples then undergo an 18 hour period of forced aeration to remove the ethylene oxide gas that has leeched into the polymer. The entire process takes 41 hours and results in effective sterilized polymer as well as a need to dispose of the toxic gas.

2.2.3 Gas Plasma

Low-temperature gas plasma has only been around since the early 1990's but is quickly becoming a preferred sterilization method due to the low temperature conditions and the environmentally friendly reagents. The two commercially available methods deactivate microorganism by ionizing either peracetic acid or hydrogen peroxide to create low temperature gas plasma. The sterilization process can be accomplished at temperatures lower than 50ºC and cycle time for this process can range anywhere from 75 minutes to 3-4 hours. Because of the lack of hazardous byproducts and short cycle times, this method is the most economically desirable approach. The gas plasma sterilization affect on the chemical and mechanical properties of the UHMWPE is still not well known. Some research suggests that there plasma sterilization has little affect on the physio-chemical properties while other work shows that it can induce surface oxidation of the polymer. [13,14,15,16,17]

2.3 Supercritical Carbon Dioxide (CO_2)

Supercritical fluid science deals with fluids above their critical temperature and critical pressure. After reaching the critical point, the fluid enters a supercritical phase in which there is no longer a distinction between gas and liquid phases. Supercritical fluids have solvent properties and densities similar to those normally associated with liquids but have mass transfer properties and viscosities more commonly associated with gases.[18] Carbon dioxide is often used within the supercritical region because the aforementioned

properties can be reached at relatively low critical temperature and pressure values (31.1ºC and 75 bar).

Supercritical CO_2 is also of interest for sterilization because it has been shown to have anti-microbial effects at high pressures while still being otherwise non-toxic, inflammable, non-hazardous, generally chemically inert, and cheap.[19,20,21 22] Although the mechanism of CO_2 sterilization is not well understood it is predominately thought of as a series of synergistically contributing factors. The increased diffusitivity properties of supercritical CO_2 allow it to cross the cellular membranes of microbes and extract necessary nutrients. The CO_2 also has the potential to react with water within the microbe and form carbonic acid. The acid lowers the internal pH of the cell and deactivates pH sensitive cellular components. The rapid entrance and exit of CO_2 (rapid pressurization[23] and depressurization[24]) from the cell could also disturb the cellular membranes and destroy the ionic gradient necessary for cell survival.

The addition of small amounts of modifiers to supercritical CO_2 has been shown to aid in the inactivation of microbes such as bacteria and fungi.[25] Work performed by Hemmer *et al*. showed that while supercritical CO_2 could inactivate bacterial resistant spores in 25 min. while at 300 atm and 110ºC; complete inactivation could be achieved in 1 hour and 40ºC with the addition of <100 ppm of hydrogen peroxide.[26] The work of White *et al*. demonstrated similar deactivation results using trifluoroacetic acid and peracetic acid. In her study, the sterilization of bacteria using peracetic was found to be 100x more

effective when pressurized using CO_2 rather than air. This demonstrates the specific synergistic effect of CO_2 + modifiers.[27]

3. Experimental

3.1 Experimental Materials and Equipment

The materials and equipment used throughout this experiment are listed below:

1. Ultra-High Molecular Weight Polyethylene Powder (GUR 1050) supplied by Ticona in Bayport, TX (average MW = 4-6 million)
2. Carbon Dioxide (food grade, 99.9% purity) purchased from BOC
3. Kimwipe® tissue paper
4. Ethanol (99.9%)
5. Hydrogen peroxide (30% in water, Ajax fine chemical pty ltd)
6. Sabouraud-4% Dextrose Agar for microbiology (1.05438.0500) was supplied by Merck in Darmstadt, Germany
7. Sodium Chloride Peptone Solution (CM0982) supplied by Oxoid in Hampshire, England
8. Tryptone Soya Agar (CM0131) supplied by Oxoid in Hampshire, England
9. White American Ginseng (Panax *quinquefolius*) that was donated by The Simply Ginseng Company in Bungendore, Australia and Natto
10. ISCO High Pressure 500D Series Syringe Pump
11. Sterile Petri Dishes
12. Metal Loop for plating
13. Bio-Rad BR-2000 Vortexer
14. 1mL pipette tips
15. Water bath and heater
16. 10mL pipette

17. Gelaire® Biological Safety Hood
18. Techno-Plas 75mL sterile containers
19. Blue Max™ 50mL Polypropylene conical tube
20. Stratos Biofuge Centrifuge
21. Thermoline Refrigerated Incubator
22. Siemens Validator® Plus AF Autoclave
23. Bacto Autoclave Waste Bag
24. 1000µL Transferpette® S pipette
25. 100µL Transferpette® S pipette
26. 1mL calibrated pipette
27. Swagelok stainless steel fittings
28. A Geo. E. & Son compression molder
29. High pressure vessel (40 mL)

3.1.1 Buffered Sodium Chloride Peptone Solution Creation

The Buffered Sodium Chloride Peptone Solution was created by mixing 14.63g of Buffered Sodium Chloride Peptone powder in 1L of distilled water. The solution was mixed for five minutes and then autoclaved at 121ºC and 0.115MPa for fifteen minutes. The solution was stored at room temperature and used when required.

3.1.2 Tryptone Soya Agar Creation

The Tryptone Soya Agar was created by mixing 40g of Tryptone Soya Agar powder in 1L of distilled water. The solution was mixed and boiled until the agar powder was

dissolved. The solution was then autoclaved at 121ºC and 0.115MPa for fifteen minutes. After autoclaving, approximately 15-20mL of the sterilized solution was poured into sterile Petri dishes. The agar was cooled in a sterile fume hood until it solidified and then the dishes were capped and stored upside down at refrigerated conditions for a maximum of two weeks.

3.1.3 Sabouraud Dextrose Agar Creation

The Sabouraud Dextrose Agar was created by mixing 65g of Sabouraud Dextrose Agar powder in 1L of distilled water. The solution was mixed and boiled until the agar powder was dissolved. The solution was then autoclaved at 121ºC and 0.115MPa for fifteen minutes. After autoclaving, approximately 15-20mL of the sterilized solution was poured into sterile Petri dishes. The agar was cooled in a sterile fume hood until it solidified and then the dishes were capped and stored upside down at refrigerated conditions for a maximum of two weeks.

3.1.4 Contaminated Buffer Solution Creation

Forty milliliters of the Buffered Sodium Chloride Peptone Solution was mixed with 4g of contaminated ginseng powder and vortexed for 1 min. The mixture was centrifuged for 10 min at 18°C and 1000 rpm. The fluid layer was extracted and stored for 3 days in a 37°C incubator. Seven milliliters of the amplified extract was mixed with 50mL of sterile Buffered Sodium Chloride Peptone solution and stored in a 37°C incubator. This solution was used to contaminate all polymer samples.

3.2 Ultra-high Molecular Weight Polyethylene

3.2.1 UHMWPE Sample Creation

Ultra high molecular weight polyethylene samples were made from powdered UHMWPE (Ticona GUR 1050). Four hundred milliliters of UHMWPE powder was poured into a square 25cm by 25cm Teflon® film covered mold in order to create a 4-5mm thick solid sheet. The bottom platen of a compression molder (Geo. E. & Son) was heated to 200°C and then the mold was placed between the upper and lower platens for 1.5 hours. The top platen was then heated to 200°C and 4MPa of pressure was applied to the mold. After 1.5 hours, the heat was turned off and the mold was left over night under pressure. The mold was then removed and the polymer square was cut into 9.5mm wide by 63.5mm long rectangles to comply with ASTM D 638 type V tensile bars.

3.2.2 UHMWPE Sample Contamination

The polymer samples were cleaned with a solution of detergent and distilled water, sterilized with 70% ethanol solution and placed under a sterile laminar flow cabinet (class 1) near an open flame to dry. Prior to each SC CO_2 sterilization experiment, 0.5mL of contaminated buffer solution was pipetted onto the surface of the UHMWPE samples (2 per experiment). The buffer solution was dried in a sterilized laminar flow cabinet near an open flame and the mass of contaminant was recorded.

3.3 UHMWPE Sterilization Procedure

A schematic diagram of the high pressure apparatus used for the sterilization is shown in Figure 3.1. The apparatus consists of a high pressure syringe pump (ISCO 500D), a

controlled temperature water bath (Thermoline TSB1), and a high pressure vessel (volume 40 mL).

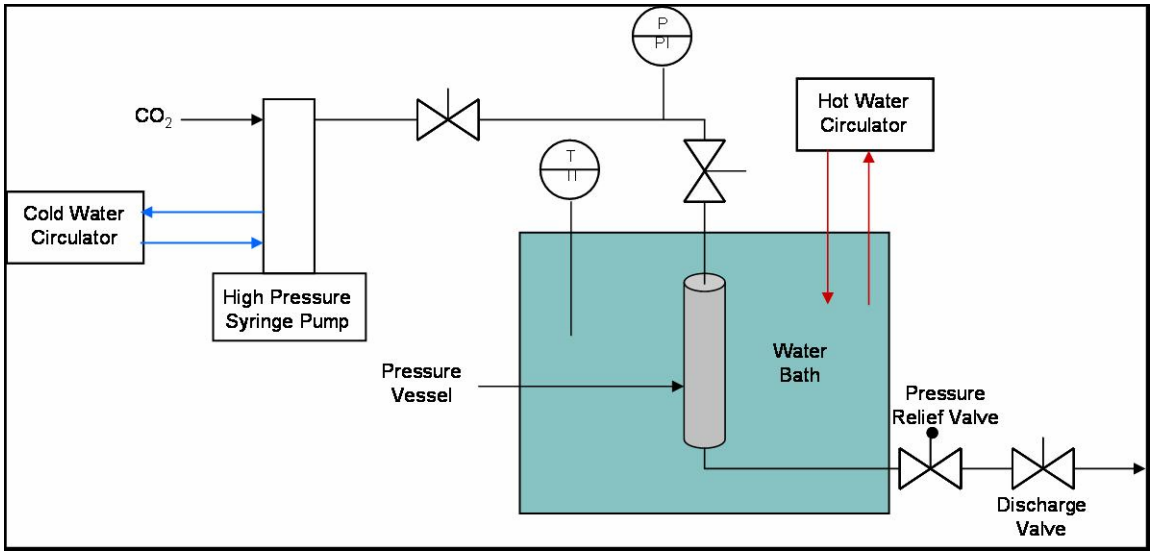

Figure 3.1: Diagram of the Sterilization Apparatus

The apparatus (pressure vessel and lines) was washed with 100wt% ethanol (EtOH) and dried overnight in an oven. It was then placed in a sterile fume hood and heated with a blow dryer to remove any excess EtOH. A Kimwipe® was sterilized using 100wt% EtOH and air dried within the sterile hood. This Kimwipe® was then placed in the lower portion of the pressure vessel and the necessary amount of modifiers (water, EtOH, and hydrogen peroxide (H_2O_2)) was pipetted onto the Kimwipe®. One contaminated sample was suspended in the upper portion of the pressure vessel and then the vessel was sealed, connected to a High Pressure ISCO Pump and submerged in a 37°C water bath. The vessel was rapidly pressurized to 170 bar CO_2 (< 5 min) for 2 hours. (The pressure, temperature, and amount of time were chosen based on a previous study in which modified CO_2 was found to completely inactivate microorganisms in contaminated ginseng at these conditions.) The vessel was then disconnected from the system, rapidly

depressurized from the bottom (< 1 minute), and moved to the sterile fume hood. The sample was removed, placed in 10mL of buffer solution, and vortexed until no contaminated solid particulate remained visible in the solution. The untreated control polymer sample was also placed in 10mL of buffer solution and vortexed in a similar manner as the sterilized sample. Two serial 100-fold dilutions were prepared for each sample by diluting 0.1mL of suspended sample in 9.9mL of autoclaved distilled water and vortexed. One milliliter from each dilution (the buffer solution in which the samples were placed and two serial dilutions) was pipetted onto a Tryptone Soya Agar plate and a Sabouraud Dextrose Agar plate. A metal loop sterilized with 100wt% EtOH and placed in an open flame was used to swab the fluid sample evenly across the surface of the agar plates. The agar plates were then covered, labeled, and placed in a 35°C incubator for 24 hours. The Tryptone Soya Agar plates were checked for bacteria and the number of colonies was recorded. The Sabouraud Dextrose Agar plates were checked for the presence of fungal growth.

3.4 Experimental Runs

The experimental conditions tested are shown in Table 3.1. All experiments were performed at 170 bar CO_2 and 37ºC for two hours. Each condition was tested twice to ensure accuracy of results.

Table 3.1: Conditions of Experimental Runs

Run	Amount of Modifier Added		
	Ethanol (mL)	Water (mL)	Hydrogen Peroxide (mL)
1	0	0	0
2	0.1	0.1	0.1
3	0.05	0.05	0.05
4	0.025	0.025	0.025
5	0.01	0.01	0.01
6	0	0	0.1
7	0	0	0.05
8	0	0	0.025
9	0.1	0	0
10	0.1	0	0.1
11	0	0.025	0.025
12	0.025	0	0.025

3.5 Microbial Measurement Procedure

The degree of sterilization of the contaminated polymer sample was expressed as a reduction of the original amount of microbes found on the contaminated control polymer sample.

The amount of bacteria was measured by the number of individual colonies that formed on the surface of the agar plates. The number of colony forming units per gram (CFU/g) was calculated using Equation 1.

$$\frac{CFU}{g} = \frac{\text{number of bacterial colonies}}{\text{Agar Plating Volume}} \times \frac{1}{\text{dilution factor}} \times \frac{\text{Volume of Contaminate}}{\text{Mass of Contaminate}} \quad \text{(Eq. 1)}^{28}$$

Where the dilution factor is 5E-2, 5E-4, or 5E-6, agar plating volume is 1mL, volume of contaminate is 0.5mL, and mass of contaminate is the measured mass of the contaminate in grams.

The CFU/g value was used in Equation 2 to calculate the total log reduction of its initial total microbial aerobic count (TAMC) due to sterilization.

$$\text{Log Reduction} = Log\left(\frac{N_0}{N_1}\right) \quad \text{(Eq. 2)}^{29}$$

Where N_0 is the number of CFU/g in the untreated contaminated sample and N_1 is the number of CFU/g in the treated contaminated sample.

Due to the nature of fungi (it grows in as one indistinguishable mass across the surface of the agar plates and thus cannot be quantified) it was recorded as a positive or negative presence. An example of bacterial and fungal growth and complete inactivation is shown in Figure 3.2.

Figure 3.2: Bacterial and Fungal Growth

Clockwise from top left: fungal growth, bacterial colonies, complete inactivation of bacteria, and complete inactivation of fungi

3.6 Experimental Validation Procedures

Before commencement of the experimentation, all solutions and sterilized items were tested to insure their sterility. Untreated contaminated polymer samples were also tested to insure a consistent level of contamination.

3.6.1 Agar Plate Validation

A random sampling of Tryptone Soya Agar and Sabouraud Dextrose Agar plates were selected from each batch of newly made agar plates and placed in a 35°C incubator for 24 hours. The agar plates were checked for the presence of bacterial and fungal growth,

respectively. No microbial growth was detected, indicating the sterile nature of the agar plates.

3.6.2 Pressure Vessel Validation

The sterilization procedure for the vessel was validated by conducting a blank run. After the sterilization of the vessel, two runs were performed using uncontaminated UHMWPE samples as controls. No microorganisms were detected on the samples after plating, corroborating that the vessel sterilization procedure was valid.

3.6.3 Sodium Chloride Peptone Buffer Solution Validation

The buffer solution was thoroughly mixed and 1mL of solution was pipetted onto three separate Tryptone agar plates and three separate Sabouraud agar plates. The plates were then placed in a 35°C incubator for 24 hours. No microbial growth was detected, corroborating that the buffer solution sterilization procedure was valid.

3.6.4 Kimwipe® Tissue Validation

The sterilized Kimwipe® was vortexed with 10mL of Peptone Buffer solution and 1mL of solution was pipetted onto three separate Tryptone agar plates and three separate Sabouraud agar plates. The plates were then placed in a 35°C incubator for 24 hours. No microbial growth was detected, corroborating that the Kimwipe® sterilization procedure was valid.

3.6.5 Autoclaved Distilled Water Validation

The autoclaved distilled water (121ºC and 0.115MPa for fifteen minutes) was thoroughly mixed and 1mL of solution was pipetted onto three separate Tryptone agar plates and three separate Sabouraud agar plates. The plates were then placed in a 35°C incubator for 24 hours. No microbial growth was detected, corroborating that the distilled water sterilization procedure was valid.

3.6.6 Contaminated Polymer Sample Validation

Two untreated contaminated samples were placed in 10mL of buffer solution and vortexed until no contaminated solid particulate remained visible in the solution. Two serial 100-fold dilutions were prepared for by diluting 0.1mL of suspended sample in 9.9mL of autoclaved distilled water and vortexed. One milliliter from each dilution (the buffer solution in which the samples were placed and two serial dilutions) was pipetted onto a Tryptone Soya Agar plate and a Sabouraud Dextrose Agar plate. A metal loop sterilized with 100wt% EtOH and placed in an open flame was used to swab the fluid sample evenly across the surface of the agar plates. The agar plates were then covered, labeled, and placed in a 35°C incubator for 24 hours. The plates were analyzed and the two separate contaminated samples were found to have similar amounts of contamination for all dilutions.

3.7 Validation Testing for the Physio-Chemical Properties of UHMWPE

3.7.1 Mechanical Strength Testing

Tensile bars were created from untreated and CO_2 + modifier treated UHMWPE samples according to ASTM D 638 type V tensile bar standards. An Instron (4468) with a 10 kN

load cell and cross-head speed of 100 mm/min was used to pull the bars until breaking. The maximum load and elongation length was recorded for each sample. These values were normalized by dividing by the sample cross-sectional area. In total, the mechanical properties of 41 tensile bars were tested (12 had not been exposed to CO_2 and 29 had been exposed).

3.7.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier transform infrared (FTIR) spectroscopy analysis was performed using a Varian 660-IR spectrometer in order to investigate the effect of CO_2 ± modifiers on the oxidation of levels of UHMWPE. The FTIR spectra for the untreated polymer sample, pure CO_2 treated polymer sample, and CO_2 + modifiers treated polymer sample were collected immediately after depressurization. The oxidation level of UHMWPE can be determined based upon normalized measurements of the area under the carbonyl peak in the region of 1650-1850 cm^{-1}.

4. Results and Discussion

4.1 Sterilization Results

The log reduction results of the experimental runs are located in Table 4.1.

Table 4.1: Log Reduction Results for Each Experimental Run

Test #	EtOH (μL)	H_2O (μL)	30 vol% H_2O_2 (μL)	EtOH (mol fract.)	H_2O (mol fract.)	H_2O_2 (mol fract.)	Bacterial log reduction	Fungal log reduction
1	0	0	25	**0.0E+00**	**1.3E-03**	**4.2E-04**	2.2	2
2	100	100	100	**2.2E-03**	**1.2E-02**	**1.6E-03**	6.0	6
3	0	0	100	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.0	6
4	0	0	100	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.0	6
5	0	0	50	**0.0E+00**	**2.6E-03**	**8.3E-04**	6.0	6
6	0	0	50	**0.0E+00**	**2.6E-03**	**8.3E-04**	6.0	6
7	100	0	0	**2.3E-03**	**0.0E+00**	**0.0E+00**	1.4	2
8	100	0	0	**2.3E-03**	**0.0E+00**	**0.0E+00**	2.1	2
9	100	100	100	**2.2E-03**	**1.2E-02**	**1.6E-03**	6.0	6
10	25	25	25	**5.6E-04**	**3.1E-03**	**4.2E-04**	6.0	6
11	25	25	25	**5.6E-04**	**3.1E-03**	**4.2E-04**	6.0	6
12	50	50	50	**1.1E-03**	**6.2E-03**	**8.3E-04**	6.0	6
13	50	50	50	**1.1E-03**	**6.2E-03**	**8.3E-04**	6.0	6
14	10	10	10	**2.3E-04**	**1.2E-03**	**1.7E-04**	3.1	2
15	10	10	10	**2.3E-04**	**1.2E-03**	**1.7E-04**	1.8	2
16	0	25	25	**0.0E+00**	**3.1E-03**	**4.2E-04**	6.0	6
17	0	25	25	**0.0E+00**	**3.1E-03**	**4.2E-04**	6.0	6
18	25	0	25	**5.6E-04**	**1.3E-03**	**4.2E-04**	6.0	6
19	100	100	100	**2.2E-03**	**1.2E-02**	**1.6E-03**	6.0	6
20	100	100	100	**2.2E-03**	**1.2E-02**	**1.6E-03**	6.0	6
21	0	0	0	**0.0E+00**	**0.0E+00**	**0.0E+00**	2.6	4
22	0	0	0	**0.0E+00**	**0.0E+00**	**0.0E+00**	1.8	2
23	0	0	100	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.0	6
24	0	0	100	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.0	6
25	0	0	25	**0.0E+00**	**1.3E-03**	**4.2E-04**	2.2	2
26	50	50	50	**1.1E-03**	**6.2E-03**	**8.3E-04**	6.0	-
27	100	100	0	**2.2E-03**	**7.2E-03**	**0.0E+00**	2.2	2.0
28	100	100	0	**2.2E-03**	**7.2E-03**	**0.0E+00**	2.2	2.0

The mole fractions were determined by using the modified Benedict-Webb-Rubin equation of state (CO_2 density determination), the volume of the high pressure vessel, the volume and density of the liquid modifiers at ambient conditions, and the respective

molecular weights of each species. The ambient condition liquid values of the modifiers were used because they were under ambient conditions during insertion and then they were pressurized.

As illustrated by Table 4.1, most of the experimental conditions tested resulted in complete inactivation. Experimental runs 2, 9-15, 19, and 20 (in which modifiers were added in equal volume amounts) demonstrate that complete sterilization can be achieved with a total modifier mole fraction of $\geq$ 4.1E-03 (Figure 4.1) which represents a total 75µL amount for all three modifiers (1:1:1 volume ratio).

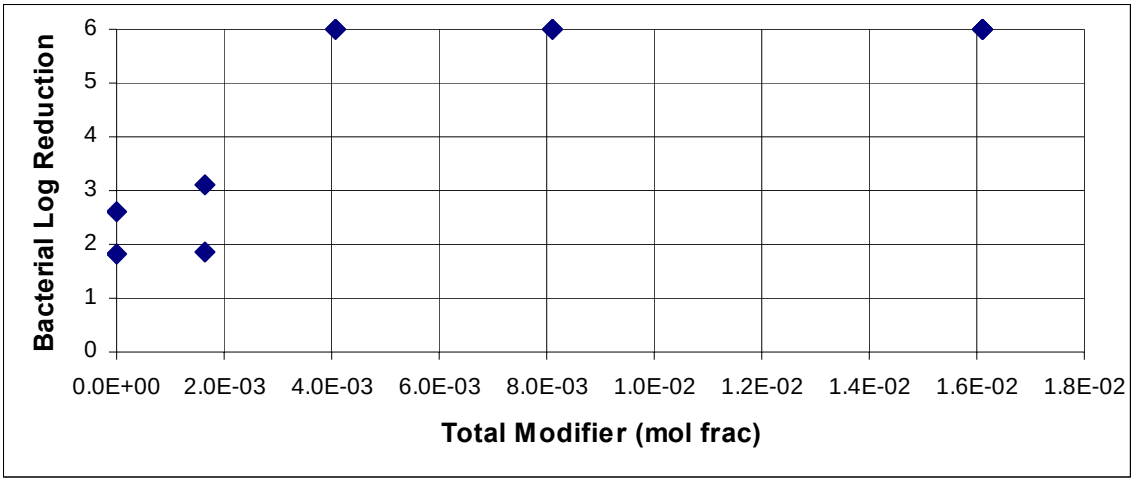

Figure 4.1: : Bacterial Log Reduction vs. Total Modifier Mole Fraction Data (Tests 2, 9-15, 19-22)

The data in Figure 4.2 shows that any mole fraction of 30vol% H_2O_2 $\geq$ 3.4E-03 (2.6E-03 water and 8.3E-04 H_2O_2 mole fractions) results in complete inactivation of microorganisms while any mole fraction $\leq$ 1.7E-03 (1.3E-03 water and 4.2E-04 H_2O_2 mole fractions) only results in a log reduction of 2.

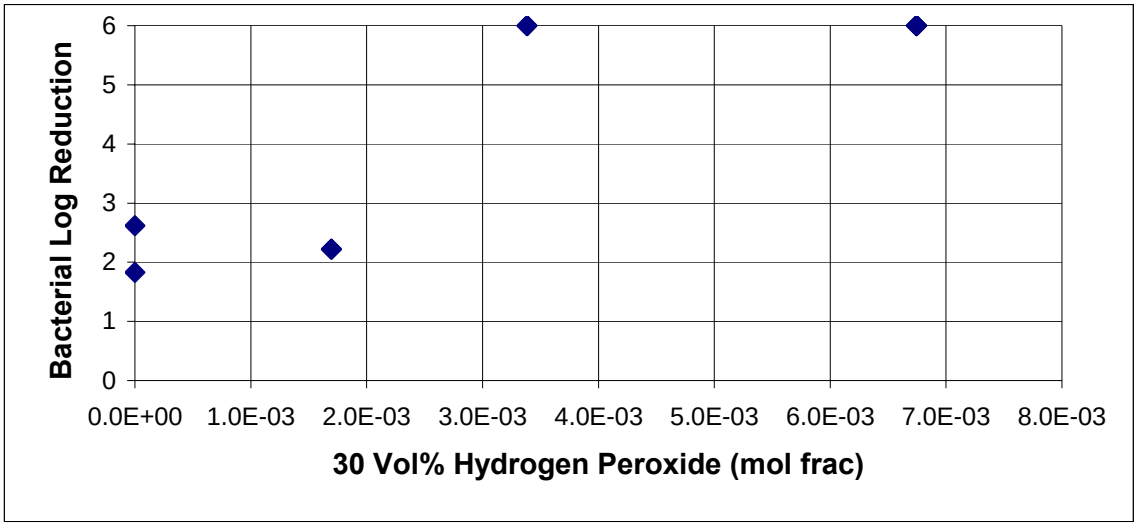

Figure 4.2: : Bacterial Log Reduction vs. 30vol% Hydrogen Peroxide Mole Fraction Data (Tests 1, 3, 4, 5, 6, 21, 22, 23, 24, 25)

The experimental data also shows that ethanol (2.2E-03 mole fraction) or an ethanol/water mixture (2.3E-03 and 7.2E-03 mole fractions, respectively) have minimal effect on the log reduction compared with CO_2 without modifiers. All three conditions yielded bacterial log reductions ranging from 1.4 to 2.6 (Tests 7, 8, 21, 22, 27, 28), whereas an ethanol/30vol% H_2O_2 mixture (5.6E-04 and 1.7E-03 mole fractions, respectively) exhibits complete sterilization (Test 18). This suggests that a synergistic sterilization effect exists due to the combination of ethanol and H_2O_2.

According to the experimental results, 30 vol% H_2O_2 has the largest effect on sterilization while ethanol, and ethanol/water combinations had negligible effects. The lowest total mole fraction of modifiers that was efficient for complete sterilization was 2.3E-03 (composed of EtOH/30vol% H_2O_2 in a 25µL:25µL ratio) and the lowest mole fraction of

a single modifier required for complete sterilization was 1.7E-03 (25μL of 30vol% H_2O_2).

In order to test for the statistically important modifier compositions an analysis of the variance (ANOVA) test (α=0.05) was run using a full factorial model. The ANOVA test shows that 30 vol% hydrogen peroxide (p=0.021), a mixture of water with ethanol (p=0.014), and ethanol with 30 vol% hydrogen peroxide (p=0.006) had the most significant impacts on the microbial log reduction. With the exception of the water ethanol mixture, the statistical results obtained corroborate well with the experimental data.

Other researchers have observed similar sterilization effects from the addition of modifiers such as water, ethanol, and H_2O_2 to SC CO_2.[30,31] Similar to this work, Dehghani *et al.* found that complete sterilization could be achieved at moderate temperatures and pressures using small mole fractions of H_2O_2. The work of Zhang *et al.* also demonstrated the increased efficacy of sterilization when using H_2O_2 as a modifier instead of water or ethanol.

4.2 Physio-Chemical Property Results

4.2.1 Mechanical Strength Results

A T-test (α=0.05) was performed on the two populations of UHMWPE tensile bars (treated and untreated) and the results demonstrated that the two populations were not statistically different (p=0.84 for $\frac{\text{maximum load}}{\text{cross-sectional area}}$ and p=0.92 for

$\frac{\text{maximum elongation}}{\text{cross-sectional area}}$). This result demonstrates that the sterilization technique used in this study had no negative effect on the mechanical strength of the UHMWPE polymer.

4.2.2 FTIR Results

The results of the FTIR analysis on the untreated polymer sample, pure CO_2 treated polymer sample, and CO_2 + modifiers treated polymer sample are shown in Figure 4.3.

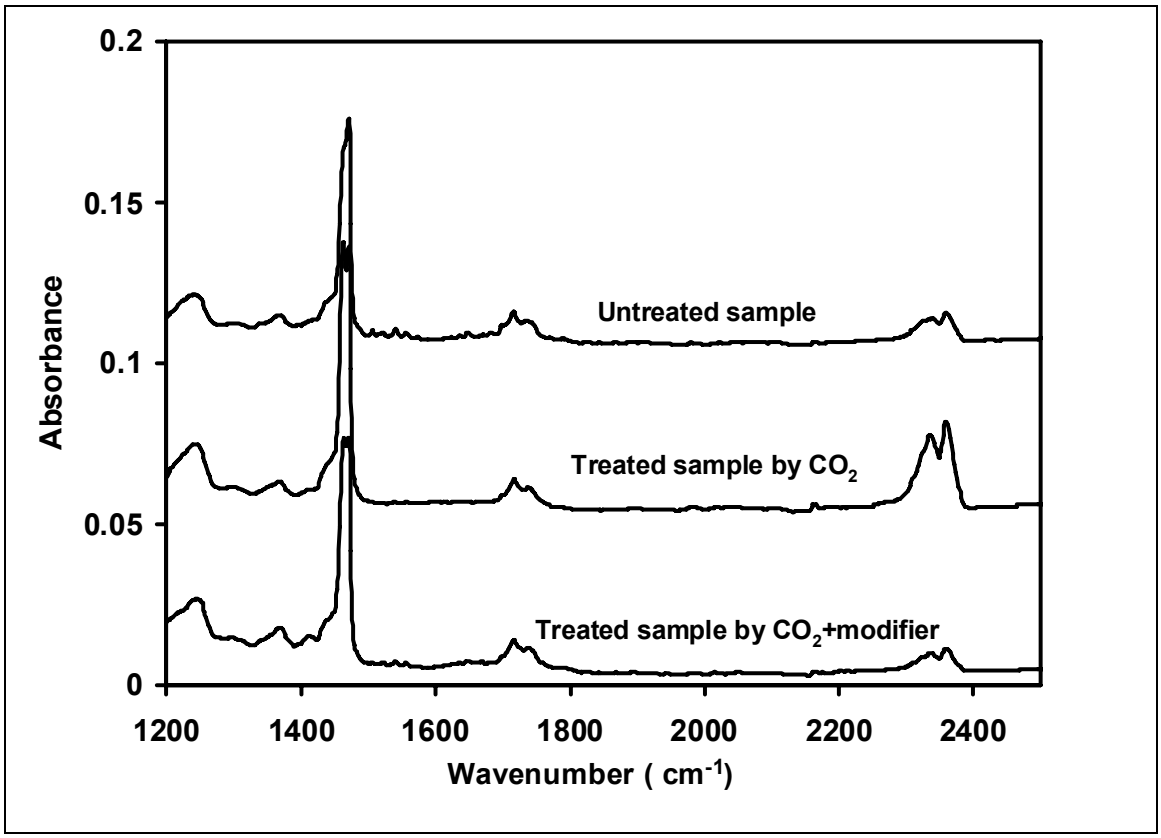

Figure 4.3: FTIR Analysis of Treated and Untreated UHMWPE

The figure demonstrates that the peak height of the carbonyl at 1715 cm^{-1}, which corresponds to the degree of oxidation, did not increase after sterilization by CO_2 or CO_2 + modifiers. This indicates that high pressure CO_2 sterilization of UHMWPE can be

accomplished without any adverse effect on the chemical integrity of the polymer. Other methods of sterilization, such as gamma irradiation and gas plasma, have been shown to produce free radicals on the surface of UHMWPE. These free radicals lead to oxidative degradation of UHMWPE, which shortens the polymer's longevity *in vivo*.[7]

4.3 Phase Behavior Modeling of CO_2 and Modifiers

In order to design better experimental procedures and understand the governing factors of CO_2 + modifier based sterilization it is crucial to determine the phase behavior of the CO_2+EtOH+H_2O+H_2O_2 quaternary system. Unfortunately, to the best of my knowledge no literature data exists for the desired experimental system so it was instead modeled as a pseudo-ternary system. This was done by assuming the moles of hydrogen peroxide could be treated as pure water. The phase equilibrium of the CO_2 + modifiers was estimated using the work of Lim *et al.* in which they studied a CO_2+H_2O+EtOH ternary system.[32] The phase diagram for the pseudo-ternary system is shown in Figure 4.4.

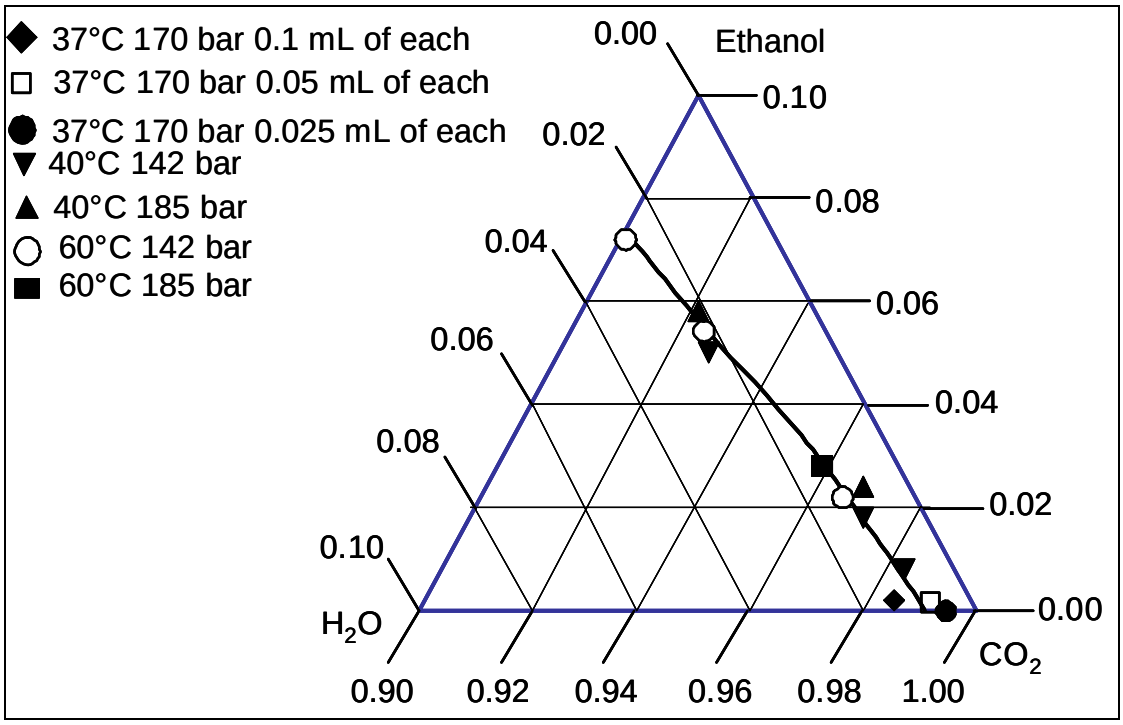

Figure 4.4: Pseudo Ternary Modeling of the High Pressure Vessel System. Data points from Lim *et al.*

The equilibrium curve in Figure 4.4 is the phase boundary that separates the one phase region (the data points to the right of the curve) and the two phase region (the data points to the left of the curve). The 37°C and 170 bar operating conditions used in this study closely matched the literature data and so the equilibrium curve for the quaternary system was assumed to be the same as the literature data ternary system. According the Figure 4.4, the quaternary data point consisting of CO_2 and 0.1mL of all three additives lies within the two phase region while the CO_2 and 0.05mL of all three additives data point lies just within the one phase region. This suggests that the CO_2 phase was saturated for the former condition and nearly saturated for the latter condition. All other experimentally tested conditions lie within the one phase region.

5. Conclusions

The complete sterilization (indicated by a 6 log reduction) of bacteria (including spores) and fungi on ultra-high molecular weight polyethylene was accomplished by using supercritical CO_2 and modifiers at 37°C and 170 bar for 2 hours. Of the three modifiers that were tested, hydrogen peroxide produced the greatest impact on bacterial and fungal log reduction, while water produced the least impact. The mechanical strength testing demonstrated that CO_2 + modifier based sterilization had no statistically significant negative effects on the tensile strength or elongation properties of the polymer. The FTIR studies showed that the sterilization process did not adversely affect the level of oxidation of the polymer.

The three aims of this study have all been met:

1) Complete sterilization of contaminated UHMWPE was achieved using CO_2 + modifiers.

2) The mechanical properties of the UHMWPE were unchanged after the sterilization process.

3) The surface of the UHMWPE showed no signs of increased oxidation.

This method of sterilization shows great promise because it uses environmentally friendly reagents at relatively safe operating conditions to achieve the desired level of sterilization. The lack of change to the physio-chemical properties of UHMWPE also makes it an excellent alternative to current sterilization because it has the ability to improve the longevity of human artificial joint implants.

6. Future Work

While the optimum conditions for sterilization of the contaminated UHMWPE samples were determined, the phase behavior of the quaternary system and the internal interactions between the CO_2 and modifiers are still not well understood. Further work needs to be done to better understand the internal workings of the system to better model the sterilization process. This can be done by either using thermodynamic simulation software to model the system or connecting the vessel to a high pressure gas chromatography unit to record the phase behavior experimentally.

Work that parallels this study should also be done on UHMWPE and metal composites to determine if the interface properties are negatively affected by the sterilization procedure. This would lead well into a one step sterilization of the entire joint replacement implant.

7. References

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APPENDICES

Table 1: Mechanical Strength Data for Polymer Samples (Load before breaking and Elongation)

Sample	Thickness (mm)	Area (mm^2)	Extension (mm)	Load (N)	Load/Area (N/mm^2)	Ext/Area (1/mm)	Crosshead Speed (mm/min)	Bag #	CO_2
1	4.31	13.79	65.1	480.5	34.8	4.7	5.08	1	yes
2	4.13	13.22	59.8	477.8	36.2	4.5	100	1	no
3	4.30	13.76	39.6	401.3	29.2	2.9	100	1	yes
4	4.00	12.80	38.4	404.0	31.6	3.0	100	1	yes
5	4.04	12.93	43.6	428.2	33.1	3.4	100	1	yes
6	4.06	12.99	64.3	535.6	41.2	4.9	100	1	yes
7	4.36	13.95	45.7	396.0	28.4	3.3	100	1	no
8	4.22	13.50	38.9	412.1	30.5	2.9	100	1	no
9	4.11	13.15	42.6	381.2	29.0	3.2	100	1	yes
10	4.14	13.25	50.6	457.7	34.5	3.8	100	1	no
11	4.15	13.28	40.3	358.4	27.0	3.0	100	1	yes
12	4.34	13.89	53.6	459.1	33.1	3.9	100	1	yes
13	4.17	13.34	42.7	413.4	31.0	3.2	100	1	no
14	3.64	11.65	71.4	508.7	43.7	6.1	100	2	yes
15	3.64	11.65	59.6	406.7	34.9	5.1	100	2	yes
16	3.65	11.68	45.8	339.6	29.1	3.9	100	2	yes
17	3.59	11.49	66.3	437.6	38.1	5.8	100	2	yes
18	3.57	11.42	45.0	365.1	32.0	3.9	100	2	yes
19	3.73	11.94	47.9	406.7	34.1	4.0	100	2	no
20	3.59	11.49	45.5	367.8	32.0	4.0	100	2	yes
21	3.70	11.84	45.0	383.9	32.4	3.8	100	2	yes
22	3.73	11.94	43.4	373.1	31.3	3.6	100	2	yes
23*	3.60	11.52	67.9	492.6	42.8	5.9	100	2	yes
24	3.70	11.84	60.0	438.9	37.1	5.1	100	2	yes
25	3.53	11.30	77.5	494.0	43.7	6.9	100	2	no
26	3.47	11.10	43.0	363.8	32.8	3.9	100	3	yes
27	3.60	11.52	69.1	452.3	39.3	6.0	100	3	yes
28	3.68	11.78	42.0	375.8	31.9	3.6	100	3	no
29	3.52	11.26	66.5	483.2	42.9	5.9	100	3	yes
30	3.70	11.84	43.8	378.5	32.0	3.7	100	3	yes
31	3.58	11.46	46.0	390.6	34.1	4.0	100	3	yes
32	3.58	11.46	67.9	496.6	43.3	5.9	100	3	yes
33	3.49	11.17	47.4	373.1	33.4	4.2	100	3	yes
34	3.64	11.65	63.9	441.6	37.9	5.5	100	3	yes
35	3.69	11.81	53.5	410.7	34.8	4.5	100	3	yes
36	3.64	11.65	44.4	378.5	32.5	3.8	100	3	yes
37	3.57	11.42	68.4	498.0	43.6	6.0	100	3	yes
38	3.55	11.36	69.1	503.3	44.3	6.1	100	4	no
39	3.52	11.26	55.5	424.2	37.7	4.9	100	4	no
40**	3.46	11.07	42.0	359.7	32.5	3.8	100	4	no
41	3.49	11.17	68.1	460.4	41.2	6.1	100	4	no

* Went into compression first accidentally

** Defect in the skinny area

Table 2: Mole Fraction Calculation Data Example

Volume of High Pressure Vessel	
Pipe OD (in)	0.75
Wall Thick (in)	0.0797
Length (in)	9
Volume (cubic inches)	2.465581
Volume (cc)	**40.40363**

Moles of Ethanol	
EtOH density (g/ml)	0.789
Volume EtOH (ml)	0.025
EtOH (g)	0.019725
MW EtOH (g/mol)	46.07
EtOH (mol)	**0.000428**

Moles of Water	
H2O density (g/ml)	1
Volume H2O (ml)	0.025
H2O2 (g)	0.025
MW H2O (g/mol)	18.0153
H2O (mol)	**0.001388**

Moles of H2O2 (30%)	
H2O2 density (g/ml)	1.44
Volume H2O2 30% (ml)	0.025
Volume H2O2 (ml)	0.0075
H2O2 (g)	0.0108
MW H2O2 (g/mol)	34.0147
H2O2 (mol)	**0.000318**
H2O density (g/ml)	1
Volume H2O (ml)	0.0175
H2O2 (g)	0.0175
MW H2O (g/mol)	18.0153
H2O (mol)	**0.000971**

Moles of CO2 present	
Temp (C)	37
Pressure (bar)	170
CO2 density (g/ml)	0.827
CO2 (g)	33.41381
MW CO2 (g/mol)	44.0095
CO2 (mol)	**0.759241**

Mole Frac	
CO2	0.99593
EtOH	0.00056
H2O	0.00309
H2O2	0.00042
Total Moles	0.76235
Total Mole Frac	1.00000

Table 3: Mole Fraction Data and Calculation Values

Test #	Ethanol (µL)	Water (µL)	H_2O_2 (30%) (µL)	Ethanol (g)	Water (g)	H_2O_2 (g)	Ethanol (mol)	Water (mol)	H_2O_2 (mol)	Total moles	Ethanol (mol frac)	Water (mol frac)	H_2O_2 (mol frac)	Total mole frac
1	0	0	25	0	0.0175	0.0108	0	0.000971	0.000318	0.76053	**0.0E+00**	**1.3E-03**	**4.2E-04**	1.7E-03
2	100	100	100	0.0789	0.17	0.0432	0.001713	0.009436	0.00127	0.77166	**2.2E-03**	**1.2E-02**	**1.6E-03**	1.6E-02
3	0	0	100	0	0.07	0.0432	0	0.003886	0.00127	0.764396	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.7E-03
4	0	0	100	0	0.07	0.0432	0	0.003886	0.00127	0.764396	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.7E-03
5	0	0	50	0	0.035	0.0216	0	0.001943	0.000635	0.761819	**0.0E+00**	**2.6E-03**	**8.3E-04**	3.4E-03
6	0	0	50	0	0.035	0.0216	0	0.001943	0.000635	0.761819	**0.0E+00**	**2.6E-03**	**8.3E-04**	3.4E-03
7	100	0	0	0.0789	0	0	0.001713	0	0	0.760953	**2.3E-03**	**0.0E+00**	**0.0E+00**	2.3E-03
8	100	0	0	0.0789	0	0	0.001713	0	0	0.760953	**2.3E-03**	**0.0E+00**	**0.0E+00**	2.3E-03
9	100	100	100	0.0789	0.17	0.0432	0.001713	0.009436	0.00127	0.77166	**2.2E-03**	**1.2E-02**	**1.6E-03**	1.6E-02
10	25	25	25	0.019725	0.0425	0.0108	0.000428	0.002359	0.000318	0.762346	**5.6E-04**	**3.1E-03**	**4.2E-04**	4.1E-03
11	25	25	25	0.019725	0.0425	0.0108	0.000428	0.002359	0.000318	0.762346	**5.6E-04**	**3.1E-03**	**4.2E-04**	4.1E-03
12	50	50	50	0.03945	0.085	0.0216	0.000856	0.004718	0.000635	0.76545	**1.1E-03**	**6.2E-03**	**8.3E-04**	8.1E-03
13	50	50	50	0.03945	0.085	0.0216	0.000856	0.004718	0.000635	0.76545	**1.1E-03**	**6.2E-03**	**8.3E-04**	8.1E-03
14	10	10	10	0.00789	0.017	0.00432	0.000171	0.000944	0.000127	0.760483	**2.3E-04**	**1.2E-03**	**1.7E-04**	1.6E-03
15	10	10	10	0.00789	0.017	0.00432	0.000171	0.000944	0.000127	0.760483	**2.3E-04**	**1.2E-03**	**1.7E-04**	1.6E-03
16	0	25	25	0	0.0425	0.0108	0	0.002359	0.000318	0.761917	**0.0E+00**	**3.1E-03**	**4.2E-04**	3.5E-03
17	0	25	25	0	0.0425	0.0108	0	0.002359	0.000318	0.761917	**0.0E+00**	**3.1E-03**	**4.2E-04**	3.5E-03
18	25	0	25	0.019725	0.0175	0.0108	0.000428	0.000971	0.000318	0.760958	**5.6E-04**	**1.3E-03**	**4.2E-04**	2.3E-03
19	100	100	100	0.0789	0.17	0.0432	0.001713	0.009436	0.00127	0.77166	**2.2E-03**	**1.2E-02**	**1.6E-03**	1.6E-02
20	100	100	100	0.0789	0.17	0.0432	0.001713	0.009436	0.00127	0.77166	**2.2E-03**	**1.2E-02**	**1.6E-03**	1.6E-02
21	0	0	0	0	0	0	0	0	0	0.759241	**0.0E+00**	**0.0E+00**	**0.0E+00**	0.0E+00
22	0	0	0	0	0	0	0	0	0	0.759241	**0.0E+00**	**0.0E+00**	**0.0E+00**	0.0E+00
23	0	0	100	0	0.07	0.0432	0	0.003886	0.00127	0.764396	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.7E-03
24	0	0	100	0	0.07	0.0432	0	0.003886	0.00127	0.764396	**0.0E+00**	**5.1E-03**	**1.7E-03**	6.7E-03
25	0	0	25	0	0.0175	0.0108	0	0.000971	0.000318	0.76053	**0.0E+00**	**1.3E-03**	**4.2E-04**	1.7E-03
26	50	50	50	0.03945	0.085	0.0216	0.000856	0.004718	0.000635	0.76545	**1.1E-03**	**6.2E-03**	**8.3E-04**	8.1E-03
27	100	100	0	0.0789	0.1	0	0.001713	0.005551	0	0.766504	**2.2E-03**	**7.2E-03**	**0.0E+00**	9.5E-03
28	100	100	0	0.0789	0.1	0	0.001713	0.005551	0	0.766504	**2.2E-03**	**7.2E-03**	**0.0E+00**	9.5E-03